CHANGES IN GROWTH, CARBOHYDRATE AND NITROGEN METABOLIC PROCESSES IN *ANABAENA FLOS-AQUAE* and *PHORMIDIUM FRAGILE* (CYANOPHYTA), UNDER THE COMBINED EFFECT OF AMITROLE AND DALAPON

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ABSTRACT. — The herbicide mixture of amitrole and dalapon appreciably attenuated the gain in dry weight of *Anabaena flos-aquae* (Lyngb.) Bréb. but stimulated the growth of *Phormidium fragile* Gom. Chlorophyll a biosynthesis was arrested in both algae. The total carbohydrates were significantly lowered in *Anabaena*, but remarkably increased in *Phormidium*, a trend that was associated with a similar rise in polysaccharide level. The mixture particularly at higher doses, increased external nitrogen secretion, and nitrate uptake. The total nitrogen content was increased in both organisms. The gain in the total soluble nitrogen fraction, was always much higher than the gain in the total insoluble nitrogen fraction.

RÉSUMÉ. — Le mélange herbicide d'amitrole et de dalapon réduit de façon appréciable la croissance (en poids sec) d'*Anabaena flos-aquae* (Lyngb.) Bréb., mais stimule celle de *Phormidium fragile* Gom. La biosynthèse de chlorophylle a est arrêtée chez les deux espèces. Les carbohydrates totaux sont diminués chez *Abaena*, mais remarquablement augmentés chez *Phormidium*, tendance associée à un accroissement similaire du niveau des polysaccharides. Ce mélange, particulièrement à hautes doses, augmente l'excrétion d'azote et l'utilisation de nitrate. L'azote total augmente chez les deux organismes. L'augmentation de la fraction azote soluble a toujours été plus élevée que celle de la fraction insoluble. (traduit par la rédaction).

KEY WORDS : herbicide mixture, *Anabaena flos-aquae*, *Phormidium fragile*, Cyanophyta.

INTRODUCTION

The profound effects exerted by applying mixtures of herbicides in weed control have attracted the attention of many researchers during recent years. Many types of mixtures have highly toxic effect on plants. The mixture composed of amitrole and dalapon gave excellent results in weed control as compared with other mixtures (KATARIA and MOOLANI, 1972; AGULHON et al.,

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Source : MNHN, Paris

1975; SINGH, 1979; MARINKOVIC et al., 1980; TURKEY et al., 1980). Interference of this mixture with the biochemistry of the treated plants had been reported by MURTAGH (1972), BOICHEV and RANGELOV (1973) and MOOLANI and KATARIA (1973). The last named authors showed that in *Cyperus rotundus*, Bladex P (dalapon 48 % and aminotriazole 32%) caused an initial decrease in protein content of plants. This was followed by a sharp increase in shoot protein content and a gradual increase in root protein content. One day after treatment, there was a decrease in the activities of the enzymes catalase and peroxidase.

The herbicide mixture, composed of dalapon and amitrole, is widely and successfully used for weed control by the Ministry of Irrigation in Egypt in the Nile and its tributaries. Hence, it was found of great interest to study the influence of this herbicide mixture on the growth and metabolism of certain local isolates of the dominant Nile phytoplankton. Little information on the effect of herbicide mixtures on microorganisms is available.

MATERIALS AND METHODS

ALGAE : The two experimental blue-green algae, namely *Anabaena flos-aquae* (Lyngb.) Bréb. and *Phormidium fragile* Gom., were isolated from El-Mohit drainage water, at sites treated with the herbicide mixture (dalapon + amitrole). These two phytoplankters were selected as being the most tolerant and predominant.

CULTURING AND TREATMENTS : In this investigation, the nutrient solution used by SHAKEEB (1975) was used for *Phormidium*, while Bold's Basal medium (BBM) (BISCHOFF and BOLD, 1963) was used for *Anabaena*. The use of two different media for the two algae was based on the data of preliminary experiments. Seven day- old axenic cultures of each species were mixed and homogenized in the automix (4000 r. p. m.) for 5 minutes and diluted twice. The starting inoculum of each organisms was adjusted to about 1×10^5 hormogonia per one ml. Five ml of the dilute mixture were transferred to 145 ml of the nutrient media which contain the following series of herbicide mixture concentrations (amitrole + dalapon) : (1.35 + 337.5),(2.7 + 675), (5.4 + 1350) and (10.8 + 2700) ppm. All flasks were aerated with sterile air (FOGG, 1942) and incubated at 28°C in a large incubator under continuous illumination (4000 lux) by fluorescent lamps. At the end of the experimental period (7 days), the algal masses were separated from the media by filtration and washed with distilled water and analysed. The algal masses were dried by further suction, and 5 replicates from each treatment as well as the control were dried at 80°C until constant weight was attained. Another set was homogenized with 10 ml borate buffer at pH 8 before centrifugation.

CHLOROPHYLL a ESTIMATION : Five replicate samples from each concentration and the control were centrifuged. Chlorophyll was extracted with

Source : MNHN, Paris

90 % acetone. The steps of analysis and calculation recommended by METZNER et al. (1965) were followed.

BIOCHEMICAL ANALYSIS : The algal masses as well as the media were analysed for the nitrogen components, whereas the tissues were further analysed for carbohydrate content. All analysis were carried out on the uncleared media or unacidified extracts, except for the carbohydrate where clearing was necessary.

Carbohydrate analysis : All the carbohydrate fractions were estimated colorimetrically. A modification of Nelson's solution (NAGUIB, 1965) was used.

Nitrogen analysis : a) Total insoluble nitrogen : The method used was recommended by NAGUIB (1969). b) Total soluble nitrogen : Determined after NAGUIB (1969). c) Free ammonia : Estimated using the Berthelot reaction (FAWCETT and SCOTT, 1960; CHANEY and MARBACH, 1962). d) Nitrate nitrogen : Determined according to PEACH and TRACY (1956). e) Free amino nitrogen : Estimated according to the method of RUSSEL (1944). f) Peptide nitrogen : The procedures of LOWRY et al. (1951) were followed in this investigation. g) Protein nitrogen : A known quantity of the dry residue, after filtration, was dissolved in a defined volume of 4 % NaOH and kept standing 24 hours, at 25°C before centrifugation. A known aliquot of the centrifugate was analysed for its protein content using the Folin phenol procedure. h) Other soluble nitrogen : This fraction was estimated by subtracting the product of peptide, amino and ammonia nitrogen from the value obtained experimentally for the total soluble nitrogen. i) Other insoluble nitrogen : By subtracting the value of protein nitrogen from that of total insoluble nitrogen, the value of other insoluble nitrogen was calculated.

RESULTS

GROWTH : Table I shows that the herbicide mixture appreciably attenuated the gain in dry weight of *Anabaena*; particularly at higher doses of the mixture.

Table I : Effect of various concentrations of herbicide mixture (dalapon + amitrole) on the dry weight of *Anabaena flos-aquae* and *Phormidium fragile* after 7 days growth (mg of algal mass/flask).

Concentration (p.p.m.)		*Anabaena flos-aquae*	*Phormidium fragile*
Control (0.0)		42.97	63.33
I (337.5 + 1.35)		34.30**	88.30**
II (675 + 2.7)		25.40**	78.63**
III (1350 + 5.4)		24.03**	76.30**
IV (2700 + 10.8)		22.87**	76.10**
LSD	5 %	4.04	3.86
	1 %	5.75	5.49

* Results significantly different from control at the 5 % level.

** Results significantly different from control at the 1 % level.

Source : MNHN, Paris

On the other hand, the mixture stimulated the growth of *Phormidium* at all concentrations used, but the degree of stimulation tended to decrease gradually as the concentration increased. Even at the highest concentrations of the mixture, *Phormidium* 7-day yield was statistically higher than that revealed by control treatment.

PIGMENTATION : Fig. 1 shows clearly that the herbicide mixture arrested remarkably the synthesis of chlorophyll a in both organisms; a trend that was furthered by increasing the level of the mixture.

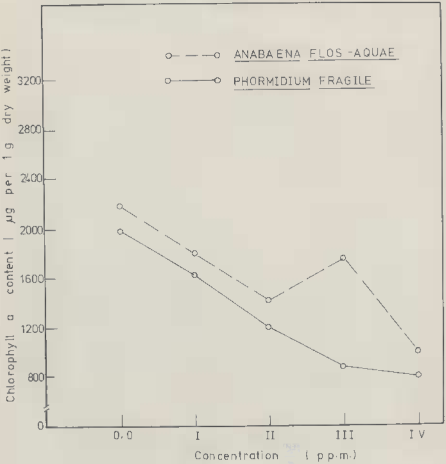

Fig. 1 : Effect of various concentrations of herbicide mixture (amitrole + dalapon) on chlorophyll a content of *Anabaena flos-aquae* and *Phormidium fragile* after 7 days growth.

Source : MNHN, Paris

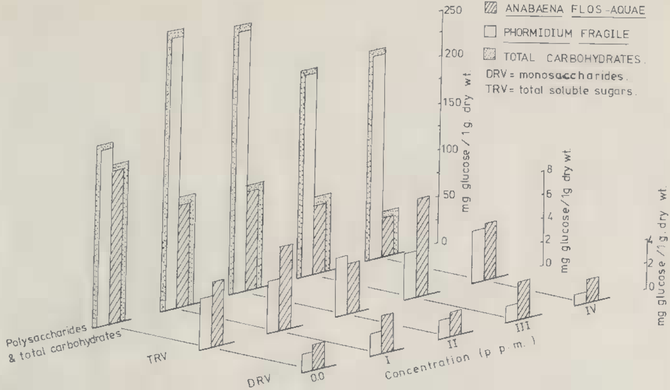

Fig. 2 : Effect of various concentrations of herbicide mixture (amitrole + dalapon) on the carbohydrate components of *Anabaena flos-aquae* and *Phormidium fragile* after 7 days growth.

Source : MNHN, Paris

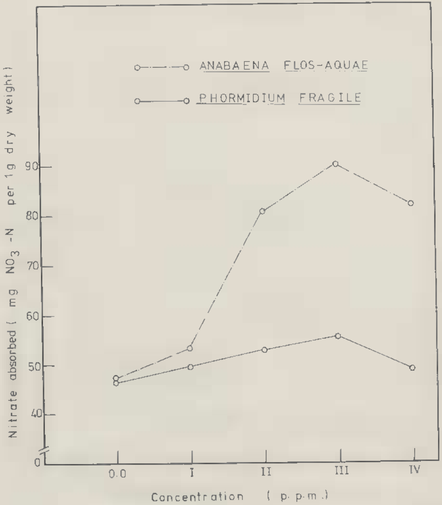

Fig. 3 : Effect of various concentrations of herbicide mixture (amitrole + dalapon) on nitrate-N absorption by *Anabaena flos-aquae* and *Phormidium fragile* after 7 days growth.

CARBOHYDRATE METABOLISM : Fig. 2 shows that the total carbohydrate content of *Anabaena*- treated cultures was significantly lowered by the herbicide mixture particularly at increased concentrations. Such a trend was accompanied by concomitant drop in polysaccharide accumulation which seemed to be the major fraction constituting the total carbohydrate content. With the highest concentration of the mixture the polysaccharide fraction dropped

Source : MNHN, Paris

to about 25 % of the control value. The herbicide mixture seemed to have minor. insignificant effects on the soluble sugar contents. In contrast to *Anabaena*, low concentrations of the herbicide mixture enhanced the accumulation of total carbohydrates in *Phormidium* (Fig. 2). This trend was associated, under all treatments mainly with increases in polysaccharide. The herbicide mixture seemed to have little, if any, effect on the soluble sugar content of *Phormidium*.

NITRATE UPTAKE : Fig. 3 shows that the herbicide mixture favoured a higher nitrate absorption by both organisms. Such a tendency was enhanced with dose increase. However, the amount absorbed by *Anabaena* tended to be significantly higher than that of *Phormidium*.

NITROGEN SECRETION IN THE MEDIA : Fig. 4 shows that the herbicide mixture appreciably increased nitrogen excretion by *Anabaena*, significantly at higher dose. Such an increase in total excreted nitrogen was mainly attributed to ammonia, peptide and/ or amino, whereas the «other soluble» fraction was subjected to some minor fluctuations. On the other hand, the herbicide mixture had little effect, on nitrogen excretion by *Phormidium*. The highest mixture level significantly enhanced nitrogen excretion but to a level clearly less than that of *Anabaena*. The increase in peptide and amino fractions at higher concentrations led to the observed enhancement of total nitrogen excretion at higher doses of mixture.

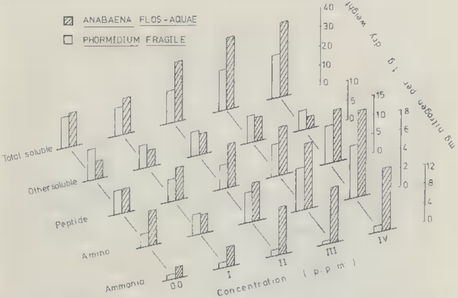

Fig. 4 : Effect of various concentrations of herbicide mixture (amitrole + dalapon) on nitrogen excretion by *Anabaena flos-aquae* and *Phormidium fragile* after 7 days growth.

Source : MNHN, Paris

Table II : Effect of various concentrations of herbicide mixture (dalapon + amitrole) on the nitrogen components of *Anabaena flos-aquae* and *Phormidium fragile* after 7 days growth (mg nitrogen per 1 g d. wt.)

Concentration (p.p.m.)	*Anabaena flos - aquae*									
	Ammonia -N	Nitrate -N	Amino -N	Peptide -N	Other soluble	T. soluble -N	Protein -N	Other insoluble	T. insoluble -N	T. N.
Control (0.0)	1.50	2.42	5.92	6.93	2.77	19.54	4.48	6.44	10.92	30.46
I (337.5 + 1.35)	1.75	4.66**	6.62*	7.41	3.58	24.03**	3.11*	8.29**	11.40	35.43**
II (675 + 2.7)	2.43*	3.69**	13.21**	7.99**	7.57**	34.89**	6.93**	9.38**	16.31**	51.20**
III (1350 + 5.4)	3.66**	4.81**	11.07**	8.74**	9.57**	37.85**	6.20**	12.97**	19.17**	57.02**
IV (2700 + 10.8)	4.50**	5.21**	8.49**	8.02**	5.52**	31.74**	5.17**	7.93*	13.10*	44.84**
LSD 5 %	0.71	0.63	0.63	0.59	1.70	1.27	1.17	1.27	2.10	2.76
LSD 1 %	1.01	0.89	0.89	0.84	2.42	1.81	1.66	1.81	2.99	3.93

Concentration (p.p.m.)	*Phormidium fragile*									
	Ammonia -N	Nitrate -N	Amino -N	Peptide -N	Other soluble	T. soluble -N	Protein -N	Other insoluble	T. insoluble -N	T. N.
Control (0.0)	0.80	2.66	4.98	5.51	4.53	18.48	3.65	7.88	11.53	30.21
I	2.08**	3.06	4.47*	6.08*	6.81**	26.17**	2.81	7.65	10.46	36.63**
II	3.28**	3.20	5.01	6.70**	5.07	23.26**	4.15*	9.83*	13.98**	37.23**
III	4.01**	3.34	4.74	5.61	1.64**	19.34	3.85	13.41**	15.26**	34.60*
IV	4.89**	1.94	4.05**	5.26	1.48**	17.62	4.88*	7.91	12.79	30.42
LSD 5 %	0.58	0.93	0.50	0.55	1.39	3.33	1.12	1.74	1.57	3.53
LSD 1 %	0.82	1.32	0.71	0.78	1.98	4.73	1.59	2.47	2.23	5.02

* Results significantly different from control at the 5 % level.
** Results significantly different from control at the 1 % level.

Source: MNHN, Paris

NITROGEN CONTENT OF THALLI : Table II shows that the herbicide mixture induced a noticeable increase in total nitrogen content of the biomass produced by both blue-green algae; a phenomenon that was enhanced with increased levels particularly in *Anabaena*. The increase was mainly in «total soluble - N» and to a lesser extent in the insoluble components, irrespective of some minor fluctuations. However, the increase of these components was clearly attenuated at the highest mixture concentration. It should be mentioned that the gain in the «total soluble nitrogen» fractions of both organisms, under all conditions, was much higher than the gain in «total insoluble -N». The increase in the total soluble fractions was mainly due to appreciable increases in ammonia, amino, peptide and nitrate nitrogen in both organisms.

DISCUSSION

The herbicide mixture increased markedly the growth of *Phormidium fragile* at all concentrations; the stimulation being maximal at the lowest dose. On the other hand, it inhibited intensively the growth of *Anabaena*, as represented by the highly significant drop in dry weights that was continued by further increases in concentration. These observations indicate that the mechanism of resistance, in the two algae, towards the mixture differ. MURTAGH (1972) demonstrated the great inhibitory effects of a mixture of amitrole and dalapon on the growth of *Dolichos lablab*, *Typha angustata*, and *Cyperus rotundus*. However, the inhibitory action of the mixture seems to depend on the organ and type of plant used.

As regards the effect of the herbicide mixture on chlorophyll a content, it seemed that it adversely suppressed the pigment synthesis in *Anabaena* and *Phormidium*; an effect that was dramatically furthered by rise in concentration. Such observations are contradictory to the findings of BOICHEV and RANGELOV (1973) who reported that applying a mixture of aminotriazole and dalapon to grape vines resulted in temporary reduction in chlorophyll a and b contents, followed by a rise above the control.

The decrease in chlorophyll a in both *Anabaena* and *Phormidium* in the presence of the herbicide mixture, match only the sharp drop in dry weight of *Anabaena* and not of *Phormidium*. The increase in dry weight of *Phormidium* in presence of all doses of the herbicide mixture, an increase that was only attenuated with high concentration is most probably due to a greater accumulation of total insoluble nitrogen and protein in *Phormidium* than in *Anabaena*.

It is difficult to interpret the variability that took place in the carbohydrate metabolism in the light of the effect of herbicide mixture treatments on chlorophyll a since such application was accompanied by a drastic drop in the pigment content of both organisms especially at high doses. A possible explanation may be in differential respiration rates of the two organisms in response to the same treatment. BOICHEV and RANGELOV (1973) demonstrated that a mixture of simazine and aminotriazole or dalapon did not reduce carbohydrate balance

Source : MNHN, Paris

in the mature shoots of grape vines, but no data are available for blue-green or other algae.

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Source : MNHN, Paris